Use of angelicin and of its structural analogues for the treatment of thalassemia

The present invention relates to a novel therapeutic use of angelicin and its structural analogues as molecules capable of inducing erythroid cell differentiation and of increasing the production of gamma-globin mRNA.

The existence of substances capable of inducing the expression of the gamma-globin gene and the biosynthesis of foetal haemoglobin (HbF) in adult subjects has been known for some time (1). In the majority of cases, those substances are also capable of activating or potentiating the transcription of genes for embryonic and foetal globins in experimental model systems.

Recently, for example, numerous DNA-binding molecules have been described that have the capacity to bring about an increase in the expression of genes for gamma-globin (2). Among these there may be mentioned cisplatin and analogues of cisplatin, mithramycin, chromomycin, and tallimustine (3). Such molecules are efficient modulators of the expression of gamma-globin genes.

In human beings, activation of the transcription of genes for gamma-globins in adult subjects leads to the production of foetal haemoglobin mimicking the phenotype HPFH (High Persistence of Foetal Haemoglobin) which confers a favourable clinical picture on patients suffering from beta-thalassaemia also in homozygotic form (4). A therapy providing for the use of molecules having such activity for the treatment of patients suffering from beta-thalassaemia could therefore make those subjects less dependent on transfusion therapy (5).

The present invention is based on the need for novel modifiers of the transcription process which can be used in the treatment of beta-thalassaemia and which have a high level of induction of the expression of gamma-globin genes and at the same time a lower cytotoxic effect than reference drugs.

We have surprisingly found that angelicin - an isopsoralen derivative which has photochemotherapic activity - as well as its structural analogues, possesses such activity. In particular, it has been found that angelicin and its structural analogues are capable of potentiating the expression of the gene for human gamma-globin.

This activity is unexpected in the light of the known therapeutic uses of angelicin and of its structural analogues (6-18).

Angelicin has in fact been proposed in the literature for the treatment of psoriasis (12, 13), as an antiproliferative agent (14, 15, 16), as an antifungal agent (8), and as an anti-inflammatory agent (17).

The structural formula of angelicin is as follows:
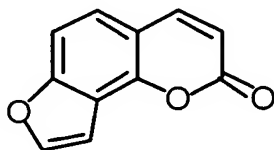
Angelicin (2-oxo-(2H)-furo-(2,3-h)-1-benzopyran)

The chemical synthesis of angelicin and its structural analogues has been described in the literature (see, for example, references 6-11).

Structural analogues of angelicin are, for example, linear and angular coumarins, optionally substituted, hetero-analogues of angelicin, thiopyrano-benzofurans, acylan-gelicins, alkylangelicins, alkoxycarbamoylangelicins, psoralens and isopsoralens, optionally substituted. A specific example is the linear furanocoumarin analogue bergapten (5-methoxypsoralen) which is currently used in PUVA (Psoralens plus UVA radiation) therapy for the treatment of psoriasis.

The structural formula of bergapten is as follows:
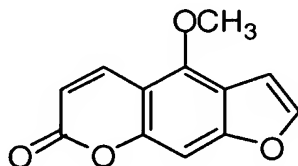
Bergapten (5-methoxypsoralen)

By virtue of their capacity to potentiate the expression of the gene for human gamma-globin, angelicin and its structural analogues can advantageously be used for the therapeutic treatment of patients suffering from beta-thalassaemia.

A first subject of the present invention is therefore the use of angelicin or a structural analogue thereof for the preparation of a medicament for the therapeutic treatment of beta-thalassaemia.

The structural analogue is preferably selected from the group consisting of linear and angular coumarins, optionally substituted, heteroanalogues of angelicin, thiopyrano-benzofurans, acylangelicins, alkylangelicins, alkoxycarbamoylangelicins, psoralens, and isopsoralens, optionally substituted.

A particularly preferred structural analogue is bergapten.

Moreover, as has been recently described (18, 19), a combined treatment with various modifiers of the transcription process permits a further increase in the expression of genes for gamma-globin.

Therefore, a second subject of the present invention is the use of angelicin or a structural analogue thereof in combination with at least one further modifier of the transcription process for the preparation of a medicament for the treatment of beta-thalassaemia.

According to a preferred embodiment, the further modifier of the transcription process is selected from the group consisting of cytosine arabinoside, retinoic acid, plicamycin, hydroxyurea, guanine, guanosine triphosphate (GTP), guanosine diphosphate (GDP) and guanosine monophosphate (GMP). Of these, cytosine arabinoside and retinoic acid are more preferred.

The activity of angelicin as an inducer of erythroid cell differentiation and the production of gamma-globin mRNA has been evaluated in human cell cultures.

The results of this study are illustrated in the following Examples. The data given in the Examples indicate that the activity of angelicin is greater than that of hydroxyurea which is a reference drug for the induction of foetal haemoglobin (HbF). It has also been ascertained that the cytotoxic effect which may be encountered is much lower than that of hydroxyurea.

The Examples which follow are provided for the purposes of illustration and are not intended to limit the scope of the invention in any way.

Example 1

The biological activity of angelicin was evaluated by examining the capacity of that compound to modulate the expression of genes for gamma-globin in the human cell line K562, which is capable of differentiating in the erythroid manner by expressing genes for gamma-globin if subjected to treatment with modifiers of the biological response that are suitable for the purpose (3, 18, 19).

The level of differentiation was evaluated by analyzing the positive reaction of the cells to benzidine (3). The production of haemoglobin was evaluated by electrophoresis on cellulose acetate and by colouring the gel with a solution based on benzidine/H_2O_2 (3). The expression of the genes encoding for gamma-globin was evaluated by quantitative RT-PCR (reverse transcriptase PCR) (3).

Some of the data obtained are given in Table 1. As will be readily appreciated, angelicin (400 µM) is capable of bringing about an increase in the percentage of K562 cells that react positively to benzidine (55-60% of the cells treated, compared with 3-4% of the control K562 cells). The chief haemoglobin produced by K562 cells treated with angelicin was Hb Portland (alpha2gamma2). The data obtained by quantitative RT-PCR demonstrate that this effect on differentiation is associated with an increase in the intracytoplasmic accumulation of mRNA for gamma-globin. These evaluations were carried out after induction with 400 µM angelicin for 6 days.

Bergapten also showed the capability to bring about differentiation (measured as increase in cells that react positively to benzidine), although this capability was associated with a lesser increase in the accumulation of mRNA for globin.

The induction of erythroid differentiation in cells treated with angelicin is very similar to that obtained with cytosine arabinoside, which is one of the most effective known inducers (18, 19). However, the activity of angelicin on the increase in production of mRNA for gammma-globin is significantly greater than that of cytosine arabinoside.

Table 1

Compound	Concentration (μM)	[a]Erythroid differentiation (%)	[b]Gamma-globin mRNA
-	-	3-4	1
cytosine arabinoside	1	75-80	3.24
bergapten	200	50-60	3.48
angelicin	400	55-60	44.94

[a] Erythroid differentiation = percentage of K562 cells that react positively to benzidine (mean ± SD of six experiments). The concentrations indicated are those that are optimal for each molecule in order to activate the erythroid differentiation. Increasing these concentrations brings about a reduction in the effect on the differentiation parameters in combination with a cytotoxic activity of the molecules themselves.

[b] The accumulation of RNA for gamma-globin is given in the Table as an increase compared with that of untreated control K562 cells. The technique used was that of quantitative RT-

PCR (22, 23) using the following primer and probe oligonu-
cleotides: gamma-globin forward primer, 5'-TGG CAA GAA GGT
GCT GAC TTC-3'; gamma-globin reverse primer, 5'-TCA CTC AGC
TGG GCA AAG G-3'; gamma-globin probe 5'-FAM-TGG GAG ATG CCA
TAA AGC ACC TGG-TAMRA-3' (FAM = 6-carboxy fluorescein, TAMRA
= 6-carboxy-N,N,N',N'-tetramethyl- rhodamine). The analyses
were carried out on material extracted from cells treated for
6 days in the conditions indicated.

Example 2

In order to check whether angelicin was also capable of
stimulating the production of mRNA for gamma-globin in human
erythroid precursors isolated from peripheral blood, the
technique published by Fibach et al. (20, 21) was used. This
technique provides for two stages: in the first stage, the
cells isolated from peripheral blood of a subject who is
healthy or suffering from a haemopoietic pathology, such as
sickle cell anaemia or beta-thalassaemia, are sown in a cul-
ture medium to which 10% of conditioned medium derived from
the vesicle carcinoma cell line 5637 has been added. The sec-
ond stage consists in cultivating the isolated cells in a
suitable culture medium, supplemented by the erythropoietin
hormone, 30% bovine foetal serum, 2-mercaptoethanol, albumin,
glutamine and desamethasone in order to permit the prolifera-
tion and maturing of stem cells of the erythroid type. In
this stage the cells can be treated with potential HbF induc-
ers. For example, with this system it was demonstrated that
hydroxyurea, an inhibitor of DNA synthesis currently used in
the experimental therapy of beta-thalassaemia, is capable of
bringing about the production of HbF.

The results obtained by this technique demonstrated an in-
crease in the production of mRNA for gamma-globin in cells
treated with angelicin compared with the same untreated cells

(20.53 times). This increase is greater than that which may be encountered using hydroxyurea (Table 2).

Table 2

Compound	Concentration (µM)	[a] Gamma-globin mRNA
–	–	1
angelicin	500	20.53
hydroxyurea	120	6.96

[a] The accumulation of RNA for gamma-globin is given in the Table as an increase compared with that of untreated control erythroid precursors. The technique used was that of quanti-tative RT-PCR (22, 23) using the primer and probe oligonu-cleotides described in Table 1. The hydroxyurea was used at a concentration of 120 µM since this molecule has a high cy-totoxic activity at concentrations comparable with those used for angelicin. This highly cytotoxic activity starts to be encountered at concentrations greater than 250-300 µM.

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